Remarks/Arguments

Amendments to the Claims:

Claim 1 was amended to more clearly define the invention. The amendment to claim 1 is supported in the specification on page 5, last 2 lines; page 6, line 11, and the paragraph spanning pages 20 and 21. No new matter has been added and entry of the claim amendment is requested.

35 U.S.C. 103:

There is a single rejection in this Office Action. Claims 1-13 stand rejected under 35 U.S.C. 103 as allegedly obvious in view of Ross (WO 91/06678) and Williams (US 6,255,083). Applicants respectfully traverse as follows:

The object of the claimed invention is directed to a "rapid, cost-effective, high throughput method for sequencing unknown nucleic acid samples" (Specification, page 3, lines 5-8). This goal is met by the method of claim 1. Briefly, claim 1 involves immobilizing a plurality of polymerases on a solid support in the absence of nucleic acid wherein each polymerase is immobilized in a reaction center of the solid support and wherein said solid support comprises a plurality of reaction centers each containing a single polymerase located at an optically resolvable distance from each other (step a). Then, a single nucleic acid sample for each of the plurality of polymerases is provided where each of the nucleic acid sample hybridizes to a single oligonucleotide primer (step b). Four different nucleotides, each nucleotide being differentially-labeled with a detachable labeling group and blocked at the 3' portion with a detachable blocking group are provided wherein the polymerase extends the primer hybridized to the nucleic acid sample with a single differentially-labeled nucleotide that is complementary to the sample nucleic acid to create a single detachable labeling group attached to the solid support (step c). After the extension of the previous step, nucleotides that are not incorporated into the primers are removed (step d) and the sample comprising the single polymerase, single nucleic acid, and the extended oligonucleotide are detected by detecting the label on the incorporated nucleotide (step e). Once the type of incorporated nucleotide has been identified (step e), the 3' blocking group and labeling group are separated from the incorporated nucleotide (step f) and removed (step g) to produce an unlabeled nucleic acid sample.

An important element of the claimed method is a confirmation steps which follows. After the 3' blocking group and labeling groups are removed, its removal from the nucleotide incorporated in the primer of each reaction center is confirmed by detecting for the presence of the single labeled nucleotide in each of the reaction centers (step h) wherein the presence of a labeled nucleotide indicates that the step of separating the labeling group from the incorporated nucleotide was not successful (step i). If the removal steps were successful (i.e., the blocking groups are removed), the steps c to h is repeated and sequence information is gathered.

The claimed invention is not obvious over a combination of Ross (WO 91/06678) and Williams (US 6,255,083) at least because these references, individually or in combination, does not disclose at least steps (a) and (h) of the claimed invention.

In contrast to Applicants claimed invention, Ross is directed to the immobilization of multiple identical copies of DNA templates and is not directed to the immobilization of polymerases wherein each polymerase is immobilized in a reaction center of a solid support and wherein said solid support comprises a plurality of reaction centers each containing a single polymerase located at an optically resolvable distance from each other - as recited in instant claim 1, step (a). Ross is directed to a method of sequencing multiple identical copies of a template nucleic acid molecule which are bound directly onto a solid support and hybridized to a sequencing primer. Ross' method involves elongating the multiple copies of the solid support bound nucleic acid in a reaction chamber in the presences of labeled nucleotides and unbound polymerase (not immobilize to any solid support) to produce an elongated primer comprising the labeled nucleotide. The label of the labeled nucleotide is then detected on the solid support or in an effluent pipe as the labels are released from the solid support to determine a DNA sequence. In short, Ross does not perform single molecule sequencing as recited in Applicants claims.

Further, Ross does not suggest monitoring for the removal of the blocking and labeling groups from the nucleotide incorporated in the primer of each reaction center by detecting for the presence of the single labeled nucleotide in each of the reaction centers (step h). In fact, Ross is completely silent on this point.

The Office Action asserts that Ross confirms "separation and removal of the 3' blocking group from the nucleotide incorporated in the primer (Ross et al. teach identifying the complement of the labeled 3'-blocked nucleotide by detecting the label attached to it (page 13, lines 1-13; page 14, lines 30-34; page 26-28), therefore, since it is the labeled group attached to

the blocking group that is detected and it is removed before detection, Ross et al. inherently teach confirming separation and removal of the blocking group from the nucleotide incorporated into the primer.)" (Office Action, page 6, lines 16-21). Applicants disagree. Applicants' claimed method, as recited in amended claim 1, step h, is directed to "confirming separation and removal of the 3' blocking group from the nucleotide incorporated in the primer of each reaction center by detecting for the presence of the single labeled nucleotide in each of the reaction centers wherein the presence of a labeled nucleotide indicates that the step of separating the labeling group from the incorporated nucleotide was not successful." Ross does not teach or suggest this step because Ross does not examine each DNA/polymerase complex (reaction center) for the presence of a label after the label is released in a deblocking step.

Applicants disagree with the Office Action's position that the position that Ross' examination of released labels by detecting labels in a "drain line" (Ross, page 14, lines 30-34) is identical to Applicants' claimed step h (Office Action, page 6, lines 16-21). First, Ross' detection method lacks specificity. In Ross, after unblocking of the incorporated nucleotide the label is detached from the immobilized polymerase reaction. The detection of this release label in Ross' "drain line" cannot determine the source of the detected label. In other words, if the solid support has more than one reaction center (i.e., more than one polymerase), the drain line would collect the effluent from more than one reaction center and it is impossible to determine the source of a detected label (i.e., from which reaction center) that is present in a drain line. Since Ross' detection method cannot determine the source of a label, it cannot determine which reaction center has failed to deblock and or release a label.

The addition of Williams does not cure the defects of Ross. Williams is directed to the detection of PPi moieties released from NTPs as a polymerase extension product is created (See, Williams, abstract). In Williams' method, nucleotides containing a quencher and a label, attached to the gamma phosphate, are used to elongate a nucleic acid template. The quencher inhibits fluorescence from the label because of its proximity to the label. When the nucleotide is not incorporated, the quencher is proximal to the label and no fluorescence is observed. As the nucleotides are incorporated during primer elongation, the gamma phosphate is released from the nucleotide and released from the growing DNA chain and detected (Williams, column 4 lines 4-22). Most importantly, unlike Applicant's claimed invention, Williams' gamma phosphate linked label is fluorescent only if it is released from the solid support because the quencher is attached to the solid support. Williams' labels, because of their proximity to the quencher,

would not be detectable if they are attached to the solid support. Therefore, like Ross, Williams provides no suggestion or description of how a single label molecule attached to a solid support can be detected or how the removal of a labeling group can be monitored by monitoring a polymerase complex attached to a solid support. Like Ross' "drain line" detection method, Williams detects labeled PPi moiety after its release. Since Williams detects the labeled PPi after its release, Williams is inherently incapable monitoring for the removal of 3' blocking group and labeling groups after elongation since in Williams' methods (1) the labeling groups are already removed during elongation (i.e., during the sequencing step) and (2) the labeling groups are undetectable before release because of its proximity to a quencher on the nucleotide. For the reasons stated above, Ross, Williams, or a combination of Ross and Williams cannot disclose all the recited steps of Applicants' claimed methods.

The withdrawal of the 35 U.S.C. § 103 rejection is requested.

CONCLUSION

Favorable action on the merits is respectfully requested. If further discussion of this case is deemed helpful, the Examiner is encouraged to contact the undersigned at the telephone number provided below, and is assured of full cooperation in progressing the instant claims to allowance.

Applicants believe no further fee is due at this time; however, the Commissioner is authorized to charge any additional fees that may be due, or to credit any overpayment, to the undersigned's account, Deposit Account No. **50-0311**, Reference Number: **18921-001 NATL** (Customer Number: **35437**).

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